

Original Article

Antimicrobial activity of fresh extract and fractions of *Fridericia chica* (Bignoniaceae) against multi-drug resistant pathogenic *Aeromonas* isolated from *Colossoma macropomum*

Potencial antimicrobiano do extrato fresco e frações de *Fridericia chica* (Bignoniaceae) frente a *Aeromonas* patogênicas multirresistentes a antimicrobianos isoladas de *Colossoma macropomum*

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Abstract

This study evaluated antimicrobial activity of fresh extract and fractions obtained from *Fridericia chica* against multidrug-resistant pathogenic *Aeromonas* spp. isolated from *Colossoma macropomum* collected on Ilha do Maranhão, Brazil. A total of 54 bacterial isolates recovered from *C. macropomum* were confirmed as belonging to genus *Aeromonas* by phenotypic and molecular analyses and classified as multidrug resistant to commercially used antibiotics. Fresh leaves of *F. chica* were subjected to maceration, followed by rotary evaporation, lyophilization, and liquid-liquid partitioning to obtain crude extract and fractions (hexane, dichloromethane, ethyl acetate, and aqueous). Total phenolic and flavonoid contents were determined using Folin-Ciocalteu assay and quantified by linear regression based on spectrophotometric absorbance. Chemical profiling of fresh extract was performed by direct infusion mass spectrometry. Antimicrobial activity was assessed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). MIC values were determined by microdilution using resazurin as viability indicator, whereas MBC values were obtained by agar plate count using spread-plate technique. Gentamicin served as a positive control in both assays. Toxicity was evaluated *in vivo* using 120 *Tenebrio molitor* larvae exposed to extract and fractions. Fresh extract (3172 ± 1.90) and ethyl acetate fraction (2605 ± 1.65) showed the highest total phenolic and flavonoid contents. Mass spectrometric analysis identified carajurone (m/z 285.0723) and carajurin (m/z 299.0876) as major constituents in fresh extract. Ethyl acetate fraction showed strongest antimicrobial activity, with MIC of $1.95 \mu\text{g mL}^{-1}$, followed by dichloromethane fraction, with MIC values ranging from 15.62 to $31.25 \mu\text{g mL}^{-1}$. Fresh extract showed MIC values ranging from 39.06 to $10,000 \mu\text{g mL}^{-1}$. Gentamicin exhibited MIC and MBC values between 3.12 and $25 \mu\text{g mL}^{-1}$. Extract and fractions of *F. chica* showed low toxicity to *T. molitor*, with 100% survival in control groups and only slight reduction at the highest concentration tested for dichloromethane fraction. Overall, ethyl acetate and dichloromethane fractions of *F. chica* demonstrated moderate to strong antimicrobial activity against multidrug-resistant *Aeromonas* spp. isolated from *C. macropomum*.

Keywords: *Aeromonas*, bacterial resistance, aquatic ecosystems, bioprospecting.

Resumo

Objetivou-se avaliar o potencial antimicrobiano do extrato fresco e frações de pariri (*Fridericia chica* (Bonpl.) L.G. Lohmann) frente a *Aeromonas* patogênicas multirresistentes a antimicrobianos isoladas de tambaquis (*Colossoma macropomum*) oriundos da Ilha do Maranhão. Para isso, foram utilizadas 54 isolados bacterianos oriundos de *C. macropomum*, confirmadas para o gênero *Aeromonas* sp. por testes fenotípicos e molecular e, multirresistentes a antibióticos de uso comercial. Para a avaliação do perfil antimicrobiano foi preparado o extrato e as frações (hexânica, diclorometânica, acetato de etila e aquosa) da folha fresca de *F. chica* pelos processos extrativos de maceração, rotoevaporação, liofilização e partição líquido-líquido. A determinação de fenólicos totais e flavonoides do extrato e frações ocorreu pelo método de Folin-Ciocalteu e regressão linear e a leitura foi realizada em espectrofotômetro e a determinação dos espectros de massa do extrato fresco foi realizada por infusão direta em espectrômetro de massas. O teste da atividade antimicrobiana dos isolados foi realizado com o extrato fresco e frações sendo determinada

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a concentração inibitória mínima (CIM) e a concentração bactericida mínima (CBM). Para a CIM, foi utilizada a resazurina como marcador para verificação da viabilidade bacteriana pelo método de diluição em microplacas e, para o CBM realizou-se o método *spread-plate* com o ágar plate count. A gentamicina foi utilizada como controle positivo tanto no CIM como no CBM. Para a avaliação da toxicidade, foi realizado o teste in vivo do extrato e suas frações, com 120 larvas de *Tenebrio molitor*. O extrato fresco ($3172 \pm 1,90$) e a fração de acetato de etila ($2605 \pm 1,65$) exibiram os maiores teores de fenólicos totais e de flavonoides totais. Na análise química do extrato fresco, foram identificados os compostos químicos Carajurona (m/z 285,0723) e Carajurina (m/z 299,0876). Quanto à CIM, a fração acetato de etila ($1,95 \mu\text{g/mL}$) e diclorometânica ($15,62$ a $31,25 \mu\text{g/mL}$) apresentaram melhor desempenho para inibir o crescimento dos 54 isolados, seguido do extrato fresco ($39,06$ a $10000 \mu\text{g/mL}$). A gentamicina apresentou na CIM e CBM, atividade antimicrobiana semelhante, variando de $3,12$ a $25 \mu\text{g/mL}$. O extrato e frações de *Fridericia chica* apresentaram baixa toxicidade em *T. molitor*, com 100% de sobrevivência nos controles e baixa redução da taxa de sobrevivência, apenas na maior concentração testada para a fração diclorometânica. Conclui-se que a fração de acetato de etila e de diclorometânica da *F. chica* demonstraram desempenho potente a moderado contra os isolados de *Aeromonas* spp. obtidos de *C. macropomum*.

Palavras-chave: *Aeromonas*, resistência bacteriana, ecossistemas aquáticos, bioprospecção.

1. Introduction

Fridericia chica (Bonpl.) L.G. Lohmann, a member of the Bignoniaceae family, is a climbing shrub native to the Brazilian Amazon. Commonly referred to as crajiru, pariri, or carajuru, this species typically reaches heights of up to two meters (Ferreira, 2005; Lohmann, 2015). Beyond its botanical profile, the plant is highly valued for its astringent and emollient properties, as well as its characteristic red pigment. This coloration is primarily attributed to 3-deoxyanthocyanins, most notably carajurin (Zorn et al., 2001), which has facilitated the plant's integration into cosmetic formulations (Barros et al., 2008).

In addition, *Fridericia chica* exhibits a broad spectrum of pharmacological activities, including antimicrobial (Mafioleti et al., 2013), anti-inflammatory, antiangiogenic, and antiproliferative effects (Michel et al., 2015). Research has also documented its wound-healing (Aro et al., 2013), antiparasitic (Miranda et al., 2017), and antioxidant properties (Siraichi et al., 2013; Martins et al., 2016; Ribeiro et al., 2018). However, despite this extensive investigation, the antimicrobial potential of *F. chica* against pathogenic *Aeromonas* spp. isolated from native fish species remains unexplored.

Among the pathogens impacting aquaculture, bacteria of the genus *Aeromonas* are widely recognized for their virulence (Sebastião et al., 2015; Gonçalves Pessoa et al., 2019). Of the 36 currently described species, at least 19 are considered emerging pathogens (Gonçalves Pessoa et al., 2019; Fernández-Bravo and Figueras, 2020). Among these species, *Aeromonas hydrophila* stands out as one of the most frequently isolated species in global aquaculture, infecting a wide range of fish species (Gonçalves Pessoa et al., 2019). In Brazil, the occurrence of this bacterium has been reported in native fish such as *Colossoma macropomum* (Gonçalves Pessoa et al., 2019; Leão et al., 2020; Gallani et al., 2020; Silva et al., 2024; Tooba et al., 2024).

Motile aeromonads cause motile aeromonas septicemia (MAS), a disease characterized by hemorrhagic septicemia, skin lesions, hemorrhage, and ulceration, and often associated with stress-related hyperlocomotion, high morbidity, and elevated mortality rates (Figueiredo and Leal, 2008; Tavares-Dias and Martins, 2017; Bandeira Junior et al., 2019; Gonçalves Pessoa et al., 2019; Tooba et al., 2024).

Despite the recurrence of these infections and their economic impact, no antimicrobial compounds are currently approved in Brazil for treating aeromonosis in native fish. Consequently, plant-derived natural extracts with phytogetic potential (Hashemi and Davoodi, 2011) represent promising, sustainable alternatives, such as natural antimicrobials, for bacterial disease control. Therefore, this study aimed to evaluate the antimicrobial activity of the crude extract and fractions of *F. chica* against multidrug-resistant pathogenic *Aeromonas* spp. isolated from *C. macropomum*.

2. Materials and Methods

2.1. Plant material

Leaves of *Fridericia chica* (Bonpl.) L.G. Lohmann (pariri) were collected from adult plants during the rainy season in the morning, a period associated with higher concentrations of phenolic compounds (Baskar et al., 2018). Sampling was conducted in São Luís, Maranhão State, Brazil ($2^{\circ}35'21.8''$ S, $44^{\circ}12'19.1''$ W). A voucher specimen was deposited in the collection at the Ático Seabra Herbarium, Federal University of Maranhão (UFMA), under registration number 01067.

2.2. Preparation of fresh extract and fractions of *Fridericia chica* (Bonpl.) L.G. Lohmann

After collection, fresh leaves of *F. chica* (Bonpl.) L.G. Lohmann were washed, sorted, and ground using a knife mill at the Food and Water Microbiology Laboratory (LMAA), State University of Maranhão (UEMA). Fresh leaves were used to preserve thermolabile compounds and avoid chemical transformations associated with prior drying.

Plant material was mixed with 70% ethanol (70 g of fresh ground *F. chica* leaves in 700 mL of 70% [v/v] ethanol) and macerated for seven days with daily homogenization. The extract was then filtered, concentrated using a rotary evaporator at 45°C , and lyophilized. For fractionation, 10 g of fresh extract were suspended in 100 mL of distilled water, followed by liquid-liquid partitioning with organic solvents (hexane, dichloromethane, and ethyl acetate) in increasing order of polarity. The remaining aqueous phase after successive partitions constituted aqueous fraction.

Concentrations were calculated based on lyophilized material obtained from fresh extract and fractions, and ranged from 1,000 to 0.98 µg mL⁻¹. For samples that did not show minimum inhibitory concentration (MIC) within this range, assays were repeated at concentrations from 1,250 to 10,000 µg mL⁻¹.

Fractionation procedures were conducted at Microbiology Laboratory of Food and Water Control Program, Federal University of Maranhão (UFMA). All extraction and fractionation steps were performed under light-protected conditions and controlled temperature to minimize chemical degradation of secondary metabolites. After separation of fractions, extraction yields of fresh extract and individual fractions were calculated according to the Equations 1 and 2 described below:

$$\text{Total mass} = \frac{\text{Mass per mL} \times \text{Filtrate volume}}{1 \text{ mL}} \quad (1)$$

$$\text{Yield (\%)} = \frac{\text{Total mass} \times 100}{\text{Mass of material used for maceration or partitioning}} \quad (2)$$

The extract and its fractions were solubilized in 1% dimethyl sulfoxide (DMSO) for pharmacological assays.

2.3. Quantification of total phenolics and flavonoids

Total phenolic content in fresh extract and fractions of *F. chica* was determined using the Folin-Ciocalteu method, following procedures described by Waterhouse (2012). Total flavonoid content was quantified by linear regression using a quercetin standard curve at concentrations ranging from 5 to 50 µg mL⁻¹.

For flavonoid analysis, 2 mL of fresh extract were mixed with 2 mL of 2% (w/v) aluminum chloride solution and incubated in the dark for 10 min. Absorbance was then measured at 425 nm using a spectrophotometer against a blank containing 2 mL of purified water and 2 mL of 2% aluminum chloride solution. All analyses were performed in triplicate.

2.4. Direct injection mass spectrometry

A stock solution of the extract in methanol was prepared and analyzed by direct infusion using a Bruker Impact mass spectrometer equipped with electrospray ionization (ESI). Analyses were conducted primarily in positive ion mode. Source conditions were set as follows: nebulizer pressure of 12 psi, dry gas flow of 4 L min⁻¹ at 180 °C, and capillary voltage of 4500 V.

After spray stabilization, continuous signal acquisition was initiated, enabling direct detection of ions present in the sample without chromatographic separation. Full-scan mass spectra were acquired in both positive and negative ionization modes, allowing detection of molecular ions, adducts, and relevant isotopic patterns. Data acquisition and processing were performed using Bruker software.

Direct infusion mass spectrometric analyses were conducted at the Analytical Centre of the Institute of Chemistry, University of São Paulo (USP).

2.5. Bacterial isolates of *Aeromonas* spp.

Fifty-four bacterial isolates from culture collection of the Food and Water Microbiology Laboratory, State University of Maranhão (UEMA), were used in this study. All isolates were identified at genus level as *Aeromonas* spp. by phenotypic and molecular analyses. Species-level identification was performed for all isolates by molecular detection of genes encoding virulence factors, including aerolysin (*aerA*) and cytotoxic enterotoxin (*act*).

All isolates showed simultaneous resistance to three or more antimicrobial classes, according to Santos et al. (2014): Penicillins (penicillin G, oxacillin, and ampicillin associated with sulbactam), Cephalosporins (cefepime, cefoxitin, cefadroxil, and ceftriaxone), Aminoglycosides (neomycin), Quinolones (ofloxacin), and Macrolides (azithromycin). Motile *Aeromonas* reference strains obtained from American Type Culture Collection (ATCC) were included to ensure standardization and reproducibility of results.

Bacterial isolates were recovered from kidney and liver samples of *Colossoma macropomum* (36.6 ± 4.32 cm total length and 998 ± 248.30 g body weight) reared on rural farms located on Ilha do Maranhão. All farms shared similar production characteristics: (i) semi-intensive culture systems in excavated ponds; (ii) use of balanced commercial extruded feed; and (iii) tambaqui as the primary cultured species.

Before antimicrobial assays, isolates were purified on blood agar to confirm viability and absence of contamination, thereby ensuring reliability of subsequent analyses.

2.6. Determination of Minimum Inhibitory Concentration (MIC)

Bacterial suspensions were standardized by inoculating 24-h cultures into sterile saline solution to achieve turbidity equivalent to a 0.5 McFarland standard (approximately 1.5 × 10⁸ CFU mL⁻¹), confirmed by spectrophotometric reading at 600 nm, with absorbance values ranging from 0.10 to 0.15 nm. Gentamicin was used as a positive control. Plant extract and fractions were prepared as stock solutions in Mueller-Hinton broth containing 0.1% dimethyl sulfoxide (DMSO) at the following concentrations: 10,000 µg mL⁻¹ for fresh extract, hexane fraction, and aqueous fraction; 1,000 µg mL⁻¹ for dichloromethane fraction; and 250 µg mL⁻¹ for ethyl acetate fraction.

MIC assays were performed in sterile 96-well microplates. Mueller-Hinton broth (100 µL) was added to wells B-H in columns 1-9, as well as to wells in columns 10-12 (negative control) in rows A and H. Wells B-D in columns 10-12 received 100 µL of Mueller-Hinton broth (negative control), whereas wells E-G in columns 10-12 received 100 µL of gentamicin solution (positive control).

Wells A1-A9 received 200 µL of plant extract or fraction stock solutions. After homogenization, 100 µL were serially transferred from row A to row B (columns 1-9), and this procedure was repeated sequentially down to row H. After homogenization of row H, 100 µL were discarded to maintain equal volumes. Subsequently, 10 µL of bacterial suspension were added to all wells, except to negative control wells in rows A and H of columns 10-12, resulting in a final volume

of 110 µL per well. Plates were incubated at 28 °C for 24 h, the optimal temperature for growth of *Aeromonas* spp.

After incubation, 30 µL of sterile resazurin solution were added to each well, followed by incubation at 37 °C for 2 h. MIC determination was based on visual assessment: blue coloration indicated absence of bacterial growth, whereas pink coloration indicated bacterial **growth**. The MIC was defined as the lowest concentration of extract or fraction capable of inhibiting growth in at least 90% of tested bacterial isolates, corresponding to absence of color change from blue to pink.

2.7. Determination of Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration (MBC)¹ was determined using spread-plate method. This technique involved inoculating microbial suspensions onto surface of Plate Count Agar (PCA).

Aliquots of 10 µL were aseptically collected from wells used in MIC assay that showed absence of bacterial growth, indicated by blue coloration, and spread onto PCA plates. Plates were incubated at 35 °C for 24 h, after which colony-forming units (CFU) were counted. Bactericidal activity was defined as absence of colony growth or growth of up to 20 CFU on agar surface, whereas growth exceeding 20 CFU indicated bacteriostatic activity. Appearance of colonies at a given concentration indicated failure to inhibit or eliminate at least 99.9% of initial bacterial inoculum.

2.8. Toxicity assay with *Tenebrio molitor* larvae

Tenebrio molitor larvae at the final developmental stage (prepupa) were used in the toxicity assay and obtained from Biofábrica São Luís. Larvae were standardized by body weight (100-200 mg) and distributed into Petri dishes, forming 12 experimental groups with 10 larvae each, for a total of 120 individuals. Of these, nine groups were assigned to treatment conditions and three served as controls: (i) clean control (larvae and feed only); (ii) blank control (sterile H₂O); and (iii) sham control (larvae punctured with a needle without injection).

In the treatment groups, larvae received injections of 10 µL of fresh extract (100, 40, and 2.5 µg mL⁻¹), dichloromethane fraction (100, 40, and 2.5 µg mL⁻¹), or ethyl acetate fraction (100, 40, and 2.5 µg mL⁻¹). Injections were administered using an insulin syringe (30 IU), with a single needle insertion per larva.

After treatment, larvae were maintained under controlled conditions at the Iraci Paiva Coelho Entomological Collection of the Entomology Laboratory at UEMA and fed a diet consisting of oats and flaked cornmeal mixed at a 2:2:2 ratio (w/w). Survival was assessed every 24 h for seven consecutive days. Mortality was determined based on presence of melanization and absence of response to physical stimuli.

2.9. Statistical analysis

ANOVA or Kruskal-Wallis tests were used to evaluate total phenolic and flavonoid contents, as well as extraction

yields. MIC and MBC values were analyzed using descriptive statistics. Survival curve analysis of *T. molitor* larvae was performed using Kaplan-Meier method. *p* values < 0.05 were considered statistically significant. Data are expressed as mean ± standard deviation (SD). Analyses were performed using GraphPad Prism 7.0.

3. Results

3.1. Extraction yields of *Fridericia chica* (Bonpl.) L.G. Lohmann extract and fractions

The extraction method enabled recovery of fractions with different polarities through use of solvents with varying polarity. Extraction yields of fresh extract and its fractions are summarized in Table 1 and ranged from 2.46% to 19.48%.

Among the fractions obtained, the ethyl acetate fraction exhibited the highest yield, followed by the aqueous, hexane, and dichloromethane fractions (Table 1).

3.2. Total phenolic and flavonoid contents of extract and fractions from *Fridericia chica* (Bonpl.) L.G. Lohmann

Chemical quantification of extract and fractions from *F. chica* is summarized in Table 2, presenting values obtained by linear regression based on calibration curves for total phenolics ($y = 0.0007x - 0.0762$; $R^2 = 0.9940$) and total flavonoids ($y = 0.0294x - 0.0898$; $R^2 = 0.9961$).

Fresh extract ($3,172 \pm 1.90$) and ethyl acetate fraction ($2,605 \pm 1.65$) showed the highest total phenolic contents, with values significantly higher than those of the other fractions and with a significant difference between them (Table 2). A similar pattern was observed for total flavonoid content, with fresh extract ($2,606 \pm 6.13$) and, particularly, ethyl acetate fraction ($17,214 \pm 3.54$) showing the highest values and differing significantly from each other. The remaining fractions, which showed lower contents, also differed significantly from one another.

3.3. Chemical profile of the fresh extract by direct injection mass spectrometry

Analysis of *F. chica* fresh extract by mass spectrometry using positive-mode electrospray ionization (ESI⁺), performed by direct infusion and full-scan acquisition, revealed a complex chemical profile (Figure 1) characteristic of plant extracts rich in secondary metabolites. Because collision-induced dissociation (MS/MS) experiments were not performed, detected signals were interpreted as protonated molecular ions, adducts, and related ionic species, which precluded unequivocal assignment of fragment ions.

The mass spectrum obtained (Figure 1) showed a distribution of ions mainly within the *m/z* ranges of 120-260, 270-500, and 550-690, indicating presence of low-, medium-, and high-molecular-weight compounds. The most intense signal was detected at *m/z* 160.0947 and

¹ This corresponds to the lowest concentration, expressed in µg mL⁻¹, required to render microbial cells nonviable by inducing irreversible damage, resulting in 99.9% bacterial cell death.

Table 1. Extraction yield of fresh extract and aqueous, dichloromethane, hexane, and ethyl acetate fractions of *Fridericia chica* (Bonpl.) L.G. Lohmann.

Sample	Yield (%)*
Fresh extract	12.68 ^a ± 0.262
Hexane fraction	11.69 ^a ± 2.939
Dichloromethane fraction	2.46 ^b ± 0.335
Ethyl acetate fraction	19.48 ^a ± 3.332
Aqueous fraction	18.46 ^a ± 0.793

*Means of five replicates ± standard deviation are shown. Means followed by different letters within the same column differ significantly according to Tukey's multiple-comparisons test (p < 0.05).

Table 2. Total phenolic content of fresh extract and aqueous, dichloromethane, hexane, and ethyl acetate fractions of *Fridericia chica* (Bonpl.) L.G. Lohmann.

Analyzed Sample	Chemical Quantification	
	Total phenolic (mg GAE g ⁻¹)*	Total flavonoids (mg QE g ⁻¹)**
Fresh extract	3,172 ^a ± 1.90	2,606 ^a ± 6.13
Aqueous fraction	259.8 ^b ± 3.81	150.9 ^b ± 6.14
Dichloromethane fraction	1,081 ^c ± 2.52	650.5 ^c ± 6.14
Hexane fraction	703.6 ^d ± 5.04	239.8 ^d ± 4.90
Ethyl acetate fraction	2,605 ^e ± 1.65	17,214 ^e ± 3.54

*GAE = gallic acid equivalent; **QE = quercetin equivalent. Means of three replicates ± standard deviation are shown. Means followed by different letters within the same column differ significantly according to Tukey's multiple comparisons test (p < 0.05).

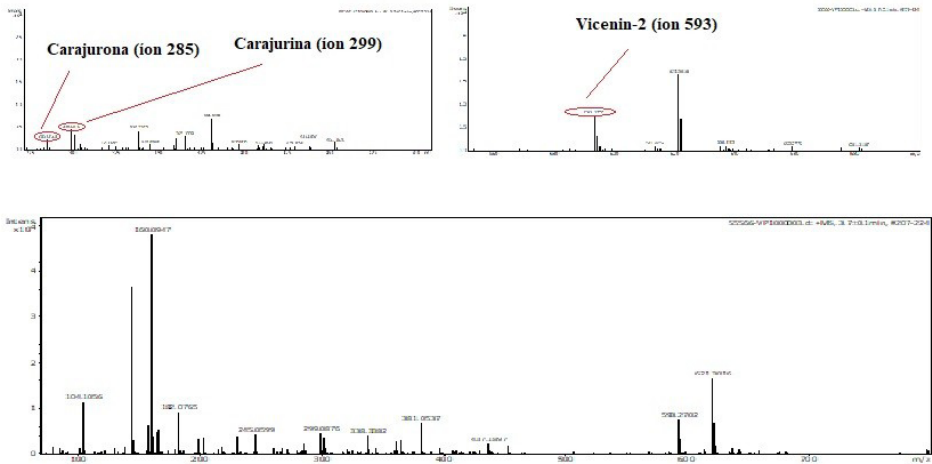


Figure 1. Mass spectrum of fresh extract of *Fridericia chica* obtained by direct infusion electrospray ionization in positive mode (ESI⁺). Source: Author's file (2025).

was interpreted as protonated molecular ion [M+H]⁺ of the major metabolite present in fresh extract. In addition to this predominant compound (m/z 160.0947), several lower-intensity ions were detected within m/z range of 130-250 (m/z 144.1001, 166.0817, 203.0501, 217.0286, and 245.0599). These signals are consistent with simple phenolic metabolites or structurally related ions commonly observed in positive-mode ESI⁺ analyses of plant extracts.

Within the intermediate mass range (m/z 280-380), prominent signals were observed at m/z 285.0723, 299.0876, 345.0552, 365.1009, and 381.0537. Based on accurate mass data, carajurone was tentatively identified at m/z 285.0723 [M+H]⁺ and carajurin at m/z 299.0876 [M+H]⁺, both previously reported in *F. chica*. Additional ions detected between m/z 400 and 460 (m/z 411.1934, 429.3693, 437.1897, and 453.1620) suggest presence of conjugated phenolic metabolites, possibly

glycosylated flavonoids or anthraquinone derivatives, indicating greater structural complexity. In the higher mass region (m/z 580-690), spectrum showed a low-complexity profile, characterized by predominance of ions at m/z 621.3016, considered the base peak in this range, and m/z 593.2702, together with lower-intensity signals at m/z 637.2959, 659.2775, and 681.3187. These ions are compatible with high-molecular-weight polyphenols, potentially diglycosylated flavonoids or poly- substituted anthraquinones, complementing metabolite profile observed at lower m/z values.

The ion detected at m/z 593.2702 is compatible, within an exploratory framework, with C-glycosylated flavonoids widely reported in plant species of the same genus, including vicenin-2 (apigenin-6,8-di-C-glucoside), whose deprotonated ion [M-H]⁻ has a theoretical m/z of approximately 594.15 (Alvarez-Ortega et al., 2021). Considering previous reports of this metabolite in *F. chica*, the detected compound may be tentatively assigned as a flavonoid structurally related to vicenin-2.

3.4. Evaluation of antimicrobial activity of *Fridericia chica* extract and fractions

Minimum inhibitory concentration (MIC) values of fresh extract and fractions from *F. chica* were determined against 54 multidrug-resistant *Aeromonas* spp. isolates, all of which were inhibited by at least one tested concentration (Table 3).

Ethyl acetate fraction showed the highest antibacterial activity against all 54 multidrug-resistant isolates tested and six *Aeromonas* spp. (*A. caviae*, *A. veronii* bv. *veronii*, *A. schubertii*, *A. sobria*, *A. hydrophila*, and *A. media*) with an MIC of 1.95 µg mL⁻¹. Its antimicrobial effect was superior to Gentamicin, as it inhibited growth of all tested isolates even at the lowest concentration evaluated.

Dichloromethane fraction also showed strong antibacterial activity, with lowest MIC value of 15.62 µg mL⁻¹ against *A. sobria* and *A. media*, but weaker activity

against *A. caviae*, *A. veronii* bv. *veronii*, *A. schubertii*, and *A. hydrophila*.

Fresh extract showed variable activity among *Aeromonas* spp. isolates, with more pronounced effects against *A. media* (39.06 µg mL⁻¹) and *A. caviae* (808.6 µg mL⁻¹). Aqueous and hexane fractions showed limited antimicrobial activity against all tested isolates, with MIC values of 10,000 µg mL⁻¹, which were markedly higher than those observed for Gentamicin (3.12-12.50 µg mL⁻¹).

MIC and minimum bactericidal concentration (MBC) results (Table 4) further confirmed strong antimicrobial potential of dichloromethane and ethyl acetate fractions of *F. chica*, as both exhibited low concentrations capable of effectively inhibiting and killing tested isolates. Notably, ethyl acetate fraction showed the best overall performance, with MIC and MBC values of 1.95 µg mL⁻¹ against all isolates, demonstrating efficacy at concentrations lower than those of Gentamicin (3.12-9.37 µg mL⁻¹), used as a broad-spectrum positive control.

MBC results also indicated strong bactericidal activity of fresh extract against *A. media* (78.12 µg mL⁻¹) and moderate activity against *A. caviae* (1,209.4 µg mL⁻¹). Overall, MIC and MBC values were closely related, indicating that fresh extract and ethyl acetate fraction of *F. chica* exerted predominantly bactericidal rather than bacteriostatic effects.

3.5. Evaluation of toxicity using *Tenebrio molitor* Larvae

Table 5 summarizes acute toxic effects of fresh extract (FE) and dichloromethane and ethyl acetate fractions of *F. chica* at different concentrations, assessed based on survival of experimental organisms over a seven-day period after treatment.

All control groups (sham, blank, and clean) showed 100% survival throughout the experiment, indicating absence of spontaneous mortality and confirming adequacy of experimental conditions. These results also demonstrate

Table 3. Minimum inhibitory concentration (MIC) of fresh extract and aqueous, dichloromethane, hexane, and ethyl acetate fractions of *Fridericia chica* (Bonpl.) L.G. Lohmann, and gentamicin, against 54 *Aeromonas* spp. isolates after 24 h of exposure.

<i>Aeromonas</i> spp.	Minimum Inhibitory Concentration - MIC (µg/mL)*					
	Fraction					
	Fresh Extract	Aqueous	Dichloromethane	Hexane	Ethyl Acetate	Gentamicin
<i>A. caviae</i> (n=30)	808.6 ± 780.3	10,000 ± 0.00	25.00± 7.66	10,000 ± 0.00	1.95 ± 0.00	4.68 ± 0.54
<i>A. veronii</i> by <i>veronii</i> (n=12)	1,660.2 ± 1,977.5	10,000 ± 0.00	27.34± 6.77	10,000 ± 0.00	1.95 ± 0.00	9.37 ± 2.75
<i>A. schubertii</i> (n=03)	1,250 ± 0.0	10,000 ± 0.00	31.25± 0.00	10,000 ± 0.00	1.95 ± 0.00	12.50 ± 0.00
<i>A. sobria</i> (n=03)	10,000 ± 0.0	10,000 ± 0.00	15.62 ± 0.00	10,000 ± 0.00	1.95 ± 0.0	6.25 ± 0.00
<i>A. hydrophyla</i> (n=03)	1,250 ± 0.0	10,000 ± 0.00	31.25± 0.00	10,000 ± 0.00	1.95 ± 0.0	3.12 ± 0.00
<i>A. media</i> (n=03)	39.06 ± 0.0	10,000 ± 0.00	15.62± 0.00	10,000 ± 0.00	1.95 ± 0.0	6.25 ± 0.00

*Means ± standard deviation.

Table 4. Minimum bactericidal concentration (MBC) of fresh extract and dichloromethane and ethyl acetate fractions of *Fridericia chica* (Bonpl.) L.G. Lohmann, and gentamicin, against 54 *Aeromonas* spp. isolates after 24 h of exposure.

<i>Aeromonas</i> spp.	Minimum bactericidal concentration – MBC (µg/mL)*			
	Fraction			
	Fresh Extract	Dichloromethane	Ethyl Acetate	Gentamicin
<i>A. caviae</i> (n=30)	1,209.4±1,102.2	35.94 ± 14.06	1.95± 0.00	4.69± 0.54
<i>A. veronii</i> by <i>veronii</i> (n=12)	2,031.3± 1,985.4	54.69 ± 13.53	1.95± 0.00	9.37± 2.75
<i>A. schubertii</i> (n=03)	2,500± 0.00	31.25 ± 0.00	1.95± 0.00	12.50± 0.00
<i>A. sobria</i> (n=03)	2,500± 0.00	62.5 ± 0.00	1.95± 0.00	6.25± 0.00
<i>A. hydrophyla</i> (n=03)	2,500± 0.00	31.25 ± 0.00	1.95± 0.00	3.12 ± 0.00
<i>A. media</i> (n=03)	78.12± 0.00	15.62 ± 0.00	1.95± 0.00	6.25± 0.00

*Means ± standard deviation.

Table 5. Acute toxicity assessment based on survival of *Tenebrio molitor* larvae over 7 days following treatment with different concentrations of fresh extract and fractions of *Fridericia chica*.

Group	Concentration (µg/mL) Tested Larvae (n°)	<i>Tenebrio molitor</i> (n= 120)		
		Live (n°)	Dead (n °)	Survival (%)
Sham control	–	10	10	100
Blank control	–	10	10	100
Clean control	–	10	10	100
FE 2.5	2.5	10	10	100
FE 40	40	10	10	100
FE 100	100	10	09	90
DM 2.5	2.5	10	10	100
DM 40	40	10	10	100
DM 100	100	10	9	90
EA 2.5	2.5	10	08	80
EA 40	40	10	09	90
EA 100				

Where: FE = fresh extract; DM = dichloromethane fraction; EA = ethyl acetate fraction.

that neither experimental procedures nor exposure medium affected larval viability.

For fresh extract, 100% survival was observed at concentrations of 2.5 and 40 µg mL⁻¹. At 100 µg mL⁻¹, one larva died, resulting in a survival rate of 90%, which indicates a slight increase in toxicity only at the highest tested concentration tested.

Dichloromethane fraction showed a toxicity profile similar to that of the fresh extract. Concentrations of 2.5 and 40 µg mL⁻¹ did not induce mortality, maintaining 100% survival. At 100 µg mL⁻¹, survival decreased to 90%, suggesting a possible dose-dependent trend, although overall toxicity remained moderate.

In contrast, ethyl acetate fraction exerted a greater effect on *T. molitor* survival than the other treatments. At 2.5 µg mL⁻¹, survival decreased to 80%, indicating increased larval sensitivity even at the lowest concentration tested. At 40 µg mL⁻¹, survival increased to 90%, approaching values observed for fresh extract and dichloromethane fraction. However, at 100 µg mL⁻¹, survival reached 100%, suggesting a non-linear toxicity response, possibly related to differences in bioavailability, metabolism, or interaction with test organism.

Survival curve analysis (Figure 2) also demonstrated absence of relevant toxicity at the lowest concentrations evaluated.

Clean group, which was neither subjected to trauma nor exposed to extracts, maintained 100% survival. The

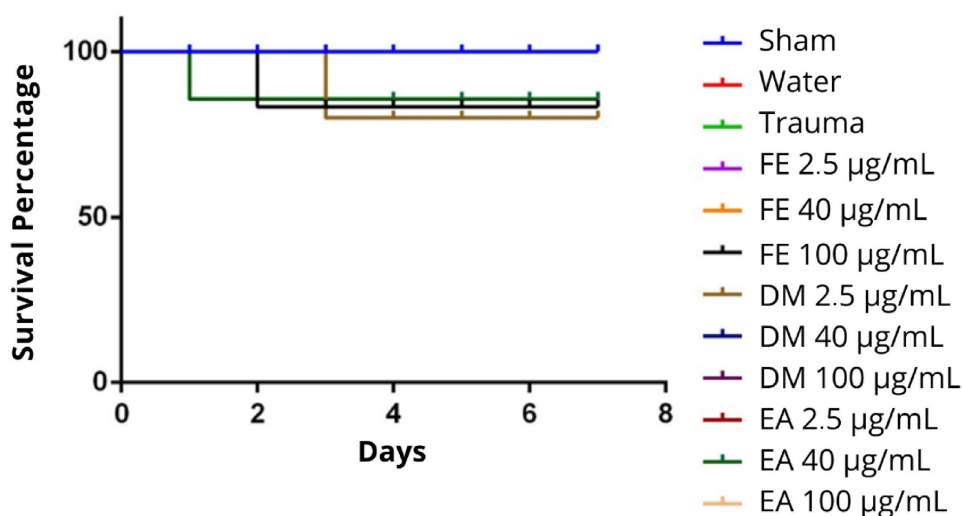


Figure 2. Survival curve analysis of *Tenebrio molitor* larvae exposed to different concentrations of fresh extract (FE) and dichloromethane (DM) and ethyl acetate (EA) fractions of *Fridericia chica*. Where: The Kaplan–Meier survival curves represent mean $\pm$ standard deviation values ($n = 10$ larvae per group).

same outcome was observed in the other control groups, indicating absence of mortality and providing a reference for normal survival.

Among groups treated with fresh extract and fractions of *F. chica*, concentrations of 2.5 and 40 $\mu\text{g mL}^{-1}$ did not affect survival, yielding results identical to those of control groups and indicating absence of detectable toxicity at these concentrations. In contrast, exposure to the highest concentration of dichloromethane fraction (100 $\mu\text{g mL}^{-1}$) reduced survival, suggesting a potential toxic effect at this concentration.

4. Discussion

The higher yield observed for ethyl acetate fraction may be related to solvent polarity, since solvents of intermediate polarity often extract a broader range of secondary metabolites. In addition, extraction yield can be directly influenced by several factors, including extraction technique, plant material type, particle size, extraction time, and temperature (Andreo and Jorge, 2006).

Likewise, the high phenolic content found in some fractions may be associated with solvent polarity, as extraction of these metabolites is generally more efficient with more polar solvents (Al-Reza et al., 2009; Mohamed et al., 2013). This relationship is supported by the low phenolic levels detected in hexane fraction, obtained with a nonpolar solvent. Although aqueous fraction represented the most polar solvent system, it showed the lowest total phenolic content, indicating that additional factors also influence extract composition. As reported by Verruck et al. (2018), variables such as soil characteristics, climatic conditions, collection period, extraction method, and chemical nature of metabolites can substantially affect phytochemical profiles.

According to Chagas (2016) and Cruz et al. (2022), flavonoids are polyphenolic compounds commonly

detected in extracts of *Fridericia chica*, especially in leaves. These compounds exhibit a wide range of biological and pharmacological activities, including anti-inflammatory, antimicrobial, analgesic, and antiviral effects. As noted by Brito et al. (2021), no single solvent can isolate all bioactive constituents present in complex plant matrices. Nevertheless, results of the present study showed that fresh extract and ethyl acetate fraction contained the highest levels of total phenolics and flavonoids.

Mass spectrometric analysis by ESI⁺ revealed marked metabolic diversity in fresh extract, particularly through detection of carajurone and carajurin, compounds recognized as chemical markers of *F. chica* (Zorn et al., 2001). Detection of these metabolites supports reliability of analytical data and agrees with previous reports describing this species as a rich source of flavonoids, especially C-glycosylated flavonoids (Siraichi et al., 2013; Campos de Siqueira and Leitão, 2019). These findings indicate consistency between chemical profile obtained in this study and profiles previously reported in the literature.

Beyond the flavonoids widely reported for this species, detection of carajurone and carajurin further clarifies chemical composition of fresh extract of *F. chica*. These compounds are recognized as chemical markers of the species, and their detection is consistent with previous reports (Zorn et al., 2001), thereby reinforcing reliability of chemical profile obtained in this study. Moreover, the presence of carajurone and carajurin is relevant because both compounds have been associated with biological activities attributed to *Fridericia chica*, further supporting robustness of phytochemical findings (Gomes et al., 2024; Silva-Silva et al., 2021; Figueiredo et al., 2023).

However, because chemical characterization was performed by direct infusion mass spectrometry in full-scan mode, current data only suggest a relationship between detected compounds (Figure 1) and antimicrobial activity (Table 3). Definitive confirmation will require

further studies involving compound isolation, structural elucidation, or MS/MS fragmentation analyses.

In this study, MIC values are consistent with those reported for medicinal plants tested against different bacterial species. For instance, Machado et al. (2003) evaluated 14 extracts of Brazilian medicinal plants and reported strong activity of *Punica granatum* L. (pomegranate) extract against methicillin-resistant *Staphylococcus aureus*. Similarly, Alzoreky and Nakahara (2003), studying *Artemisia absinthium* (absinth) extract against *S. aureus*, *Bacillus cereus*, *Listeria* spp., *Salmonella* spp., and *Escherichia coli*, reported MIC values ranging from 165 to 2,640 µg mL⁻¹. However, these concentrations were higher than those observed in present study.

Plants represent a rich source of organic and inorganic compounds with diverse biological activities and long-standing relevance to human use. Their application as complementary therapies is often based on traditional knowledge transmitted across generations or shaped by community practices (Dias et al., 2018). In a contemporary context marked by widespread and often inappropriate antibiotic use, followed by microbial adaptation to commonly used antimicrobials, Nazaré and Braz (2020) emphasized urgency of identifying new antimicrobial agents. This need is especially relevant for *Aeromonas* spp., which are major pathogens in global aquaculture and can reduce productivity while causing substantial economic losses.

Brazil has strong potential to contribute to development of phytotherapeutic products because of its biodiversity, favorable environmental conditions, and large number of medicinal plant species already identified (Brandão et al., 2006). At the same time, treatment options for bacterial diseases in fish remain limited, largely because of physiological diversity among fish species and complexity of aquatic environments, which exceed those found in terrestrial animals (Smith et al., 2008). In addition, prolonged and excessive antimicrobial use has intensified selection pressure, promoting emergence of resistant strains. This scenario is concerning because it disrupts natural aquatic microbiota, alters ecosystem dynamics, and favors proliferation of multidrug-resistant pathogens.

For these reasons, production of standardized extracts from *F. chica* with activity against pathogenic *Aeromonas* spp. isolated from tambaqui (*Colossoma macropomum*) appears promising. Results of present study demonstrate feasibility of developing phytotherapeutic products for treatment of aeromonosis in native fish species.

Molecules isolated from natural products often serve as prototypes for drug discovery and investigation of new therapeutic activities (Rates, 2001). According to Ianck et al. (2017), medicinal plants are valuable sources of bioactive compounds for pharmaceutical development and may involve lower research costs than synthetic molecules. In this context, findings of present study highlight potential of *F. chica* as an alternative strategy for control of *Aeromonas* spp. in native fish, contributing to reduced use of synthetic antibiotics and helping protect aquatic ecosystems from dissemination of antimicrobial resistance genes.

Toxicity assays using 120 *Tenebrio molitor* larvae indicated low acute toxicity, corroborating previous studies with invertebrate models. These results reinforce

potential of *F. chica* as a source of bioactive compounds with favorable safety profiles. Likewise, low or absent mortality in treated groups agrees with reports showing minimal toxicity of *F. chica* extracts in other biological models, such as *Caenorhabditis elegans* (nematode), even at elevated concentrations (Olivero-Verbel et al., 2021).

Overall, these findings indicate that plant extracts such as *F. chica* generally show low acute toxicity in invertebrate models when tested within moderate concentration ranges, with dose-dependent effects emerging mainly at higher levels. Thus, *T. molitor* is a sensitive, reliable, and reproducible model for initial toxicological screening of natural products. However, limitations of this model should be acknowledged. As an invertebrate system, it provides only an initial indication of toxicity and does not allow direct extrapolation to vertebrates or aquatic organisms. Therefore, these data should be considered a preliminary safety assessment, and further studies using vertebrate models are required to confirm safety of tested fractions.

Overall, present data underscore potential of *F. chica* as an alternative for control of *Aeromonas* spp., contributing to reduced synthetic antibiotic use in aquaculture and mitigation of antimicrobial resistance spread. Fresh extract, as well as ethyl acetate and dichloromethane fractions, showed antimicrobial activity ranging from strong to moderate against pathogenic *Aeromonas* isolates from *C. macropomum*. These findings represent an initial step in bioprospecting bioactive compounds from *F. chica*, and further studies involving compound isolation, mechanistic evaluation, and *in vivo* validation are warranted.

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Data Availability Statement

The entire dataset supporting the results of this study was published in the manuscript itself.

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